Electronic Supplementary Information

Aggregation Deaggregation Influenced Selective and Sensitive detection of Cu^{2+} and ATP by Histidine Functionalized Water-Soluble Fluorescent Perylene Diimide under Physiological Conditions and in Living Cells

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Materials and methods:

All reagents and solvents were purchased from commercial sources and the solvents used were of spectroscopic grade. UV-Vis absorption spectra were recorded on a Perkin Elmer Lambda-25 spectrometer. Fluorescence spectra were carried out on a FluoroMax®-4 Spectrofluorometer-Horiba Scientific. A 10 mm X 10 mm quartz cuvette was used for solution spectra and emission was collected at 90° relative to the excitation beam. Deionized water was obtained from Milli-Q system (Millipore). ^{1}HNMR, ^{13}CNMR were recorded on Varian AS 400MHz and Bruker 600MHz NMR spectrometers. Mass spectra were recorded by Agilent Accurate-Mass Q-TOF LC/MS 6520, and peaks are given in *m/z* (% of basis peak). FT-IR was recorded in a Perkin Elmer spectrometer with samples prepared as KBr pellets. Atomic force microscopy images were taken by Agilent 5500-STM instrument. DLS were measured by Zetasizer Nano series Nano-ZS90 instrument.

Experimental section:

Synthesis of PDI-HIS

3, 4, 9, 10-Perylenetetracarboxylic acid bisanhydride (500 mg, 1.27 mmol), histidine (800 mg, 3.82 mmol) and 2.0 g of imidazole were heated at 140 °C for 8 h with stirring. The reaction mixture was allowed to cool to 90 °C, and then poured into water. Then, the mixture

was acidified with 2.0 M HCl, and the precipitate was washed with water and dried under vacuum at 80 °C to give the product of PDI-HIS (800 mg, 94%). ^{1}H NMR (DMSO d_6, 600 MHz) δ: 8.70 (broad, 4H), 8.54 (broad, 4H), 7.45 (broad, 2H), 6.75 (broad, 2H), 5.80 (broad, 2H), 3.69, 3.53 (broad, 4H), MS (m/z): 667.1475 [M + H^+], FT-IR (cm^{-1}) 3430, 2925, 2854, 1696, 1655, 1593, 1576, 1436, 1402, 1364. ^{13}C-NMR (DMSO d_6, 600 MHz) δ: 29.18, 54.95, 116.72, 121.39, 124.49, 128.28, 131.50, 134.70, 135.09, 138.32, 154.99, 179.34.

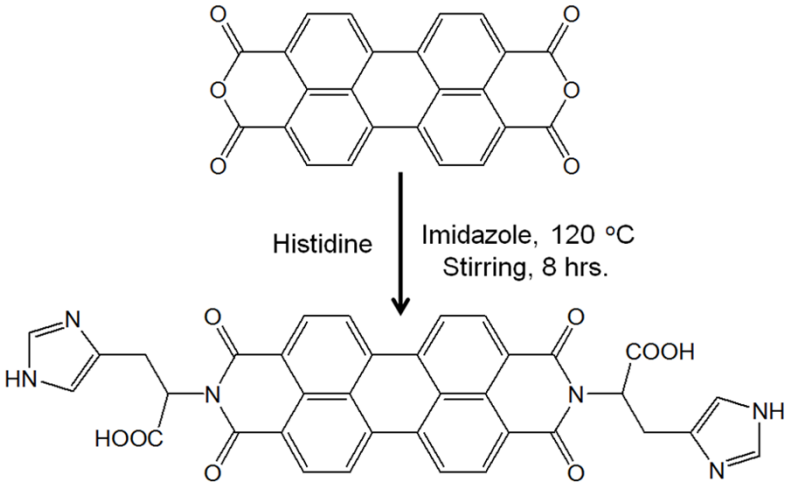

Figure S1 Synthesis of PDI-HIS. Histidine, imidazole, stirring at 120 °C, 8 hrs.

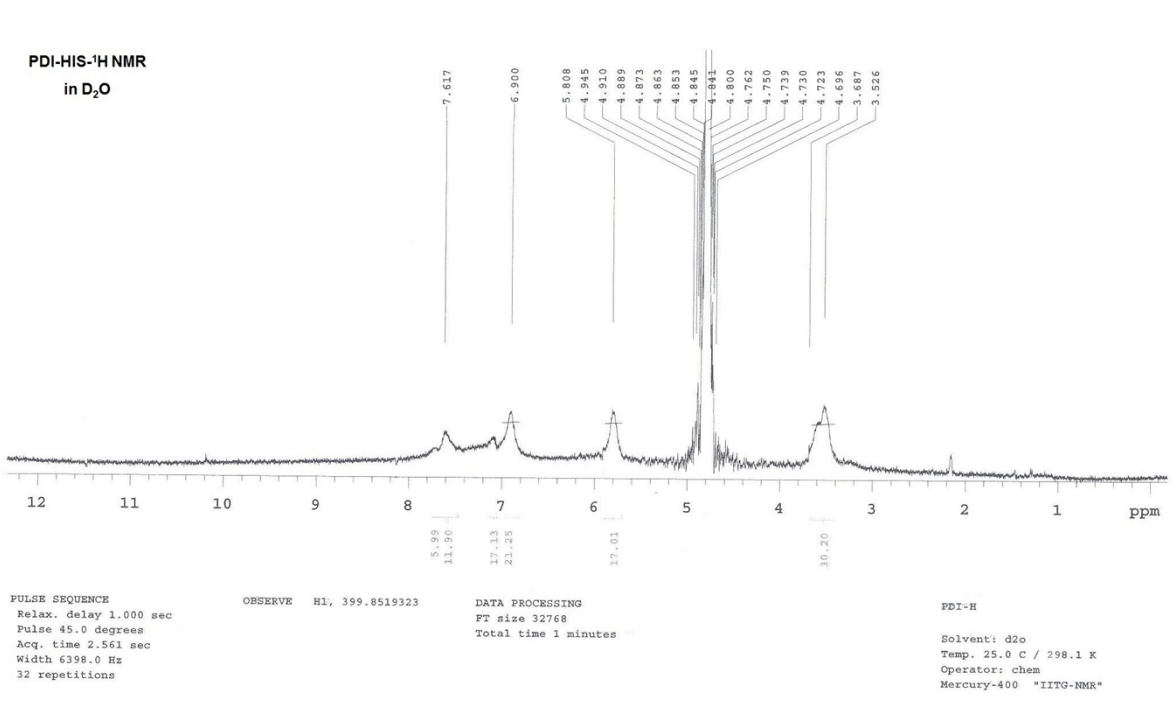

Figure S2 ^{1}H-NMR Spectra of PDI-HIS in D_2O

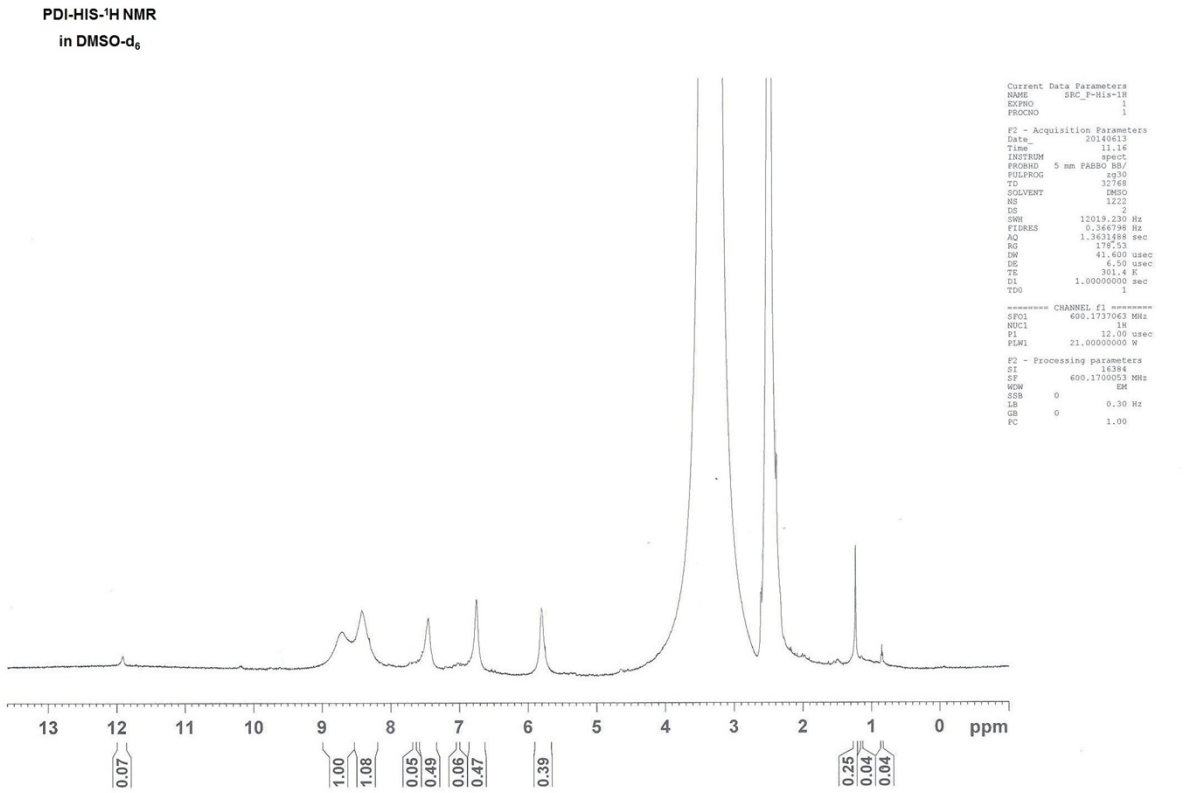

Figure S3 ^{1}H-NMR Spectra of PDI-HIS in DMSO –d_6

Preparation of stock solutions:

The PDI-HIS stock solution was prepared at a concentration of 1.0 x 10^{-3} mL^{-1} in 10 mL H_2O. This stock solution was diluted to desired concentration for each titration in 3 mL cuvette having HEPES buffer at pH 7.4.

Preparation of cation and anion stock solutions:

Each inorganic metal salt and anions stock solutions were prepared at the concentration of 10.0 x 10^{-3} mL^{-1} in 5 mL Milli-Q water. The stock solutions were diluted to the desired concentrations with Milli-Q water when needed.

Preparation of HEPES buffer solution for titration experiments:

All the UV-Visible and fluorescence titrations were performed in 10 mM HEPES buffer at pH 7.4 by using 4M NaOH or 5M HCl solution.

Proposed mechanism for PDI-HIS aggregation with Cu^{2+} and disaggregation with ATP

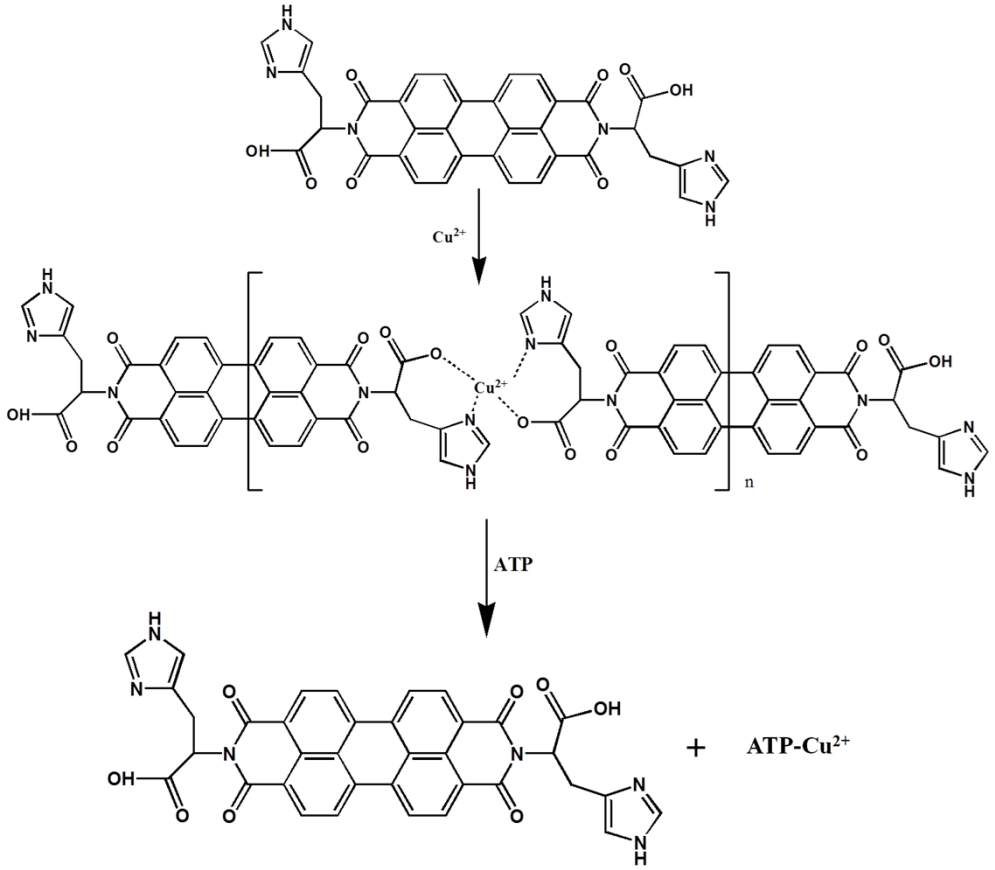

Figure S4 Proposed mechanism for PDI-HIS aggregation with Cu^{2+} and disaggregation with ATP.

Schematic representation of PDI-HIS aggregation with Cu^{2+} and disaggregation with ATP

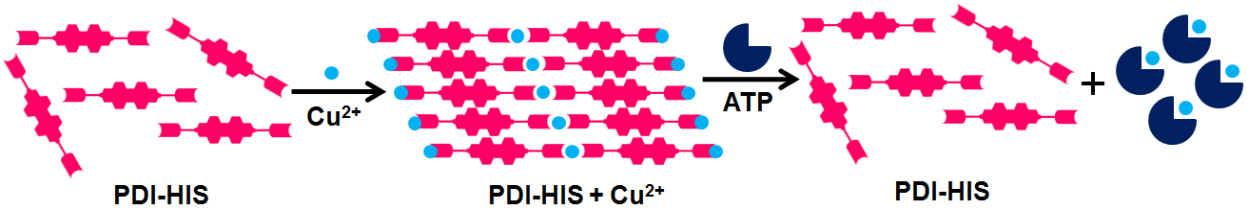

Figure S5 Schematic representation of PDI-HIS aggregation with Cu^{2+} and disaggregation with ATP.

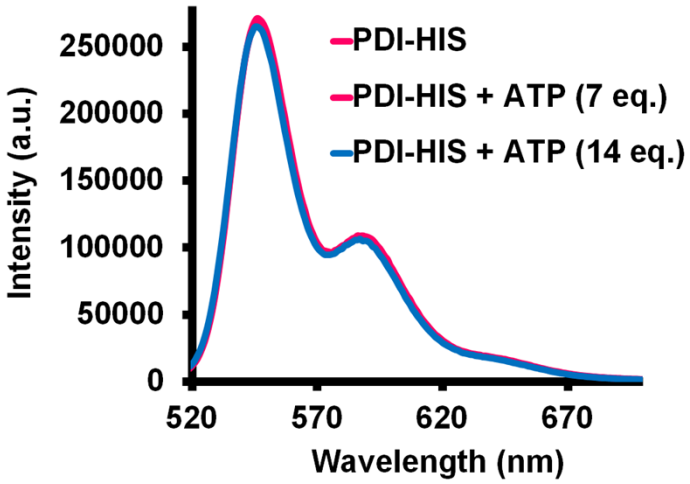

Figure S6 Fluorescence spectra of PDI-HIS (0.33 μM) observed no significant changes upon addition of ATP (4.66 μm) in HEPES buffer (10 mM, pH 7.4).

PDI-HIS is a highly selective turn-off sensor for copper ion and the association constants for the formation of PDI-HIS-Cu^{2+} complexes were evaluated using the fluorescence spectroscopy. The relative change in emission intensity of PDI-HIS at 546 nm was used to determine the binding constants.[1] The measured emission band ($I_0/\Delta I$) at 546 nm when plotted against the inverse of the concentration of Cu^{2+} solution fits almost a linear relationship. The ratio of the intercept versus slope gives the association constants of 5.65 × 10^6 M^{-1} for copper. Further, the apparent binding constant was evaluated using the Benesi−Hildebrand (B−H) plot by the fluorescence spectral changes at 546 nm for turn-on sensor for ATP with PDI-HIS-Cu complex and estimated to be 1.42 × 10^5 M^{-1}.

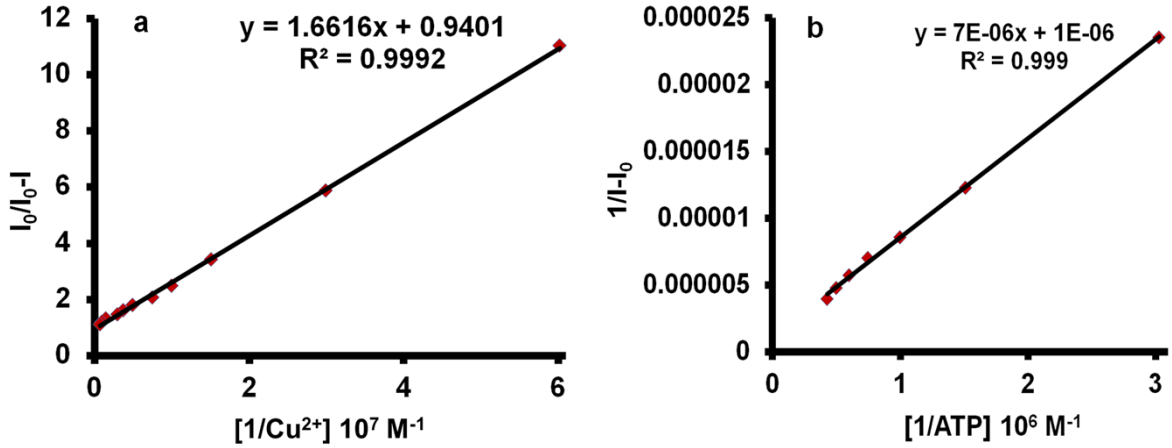

Figure S7 a) Binding constant curve for PDI-HIS with Cu^{2+} ion; b) Benesi−Hildebrand (B−H) plot for the calculation of binding constant for ATP with PDI-HIS+Cu^{2+}.

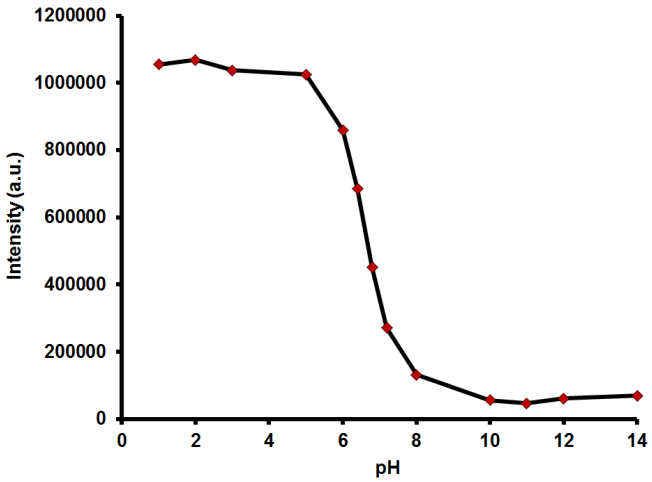

Figure S8 pH sensitivity of PDI-HIS.

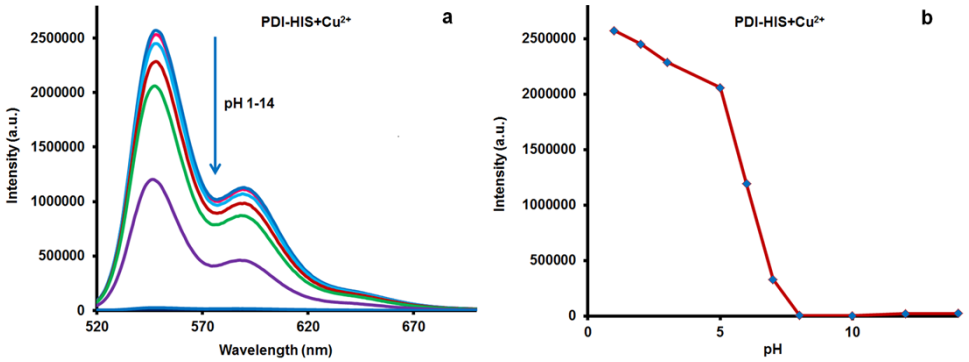

Figure S9 (a, b) Emission spectra of PDI-HIS (0.66 μm) with Cu^{2+} ions in HEPES buffer (10 mM, pH 1 to14). Excitation wavelength: 508 nm.

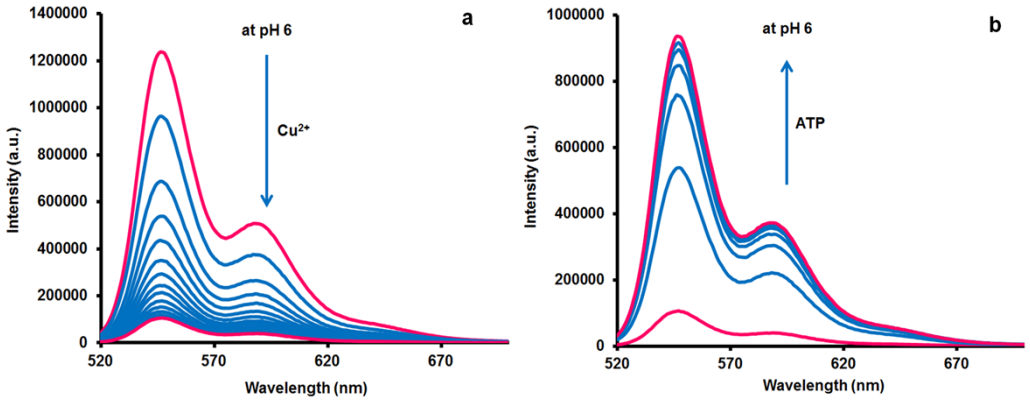

Figure S10 (a) Emission spectra of PDI-HIS (0.66 μm) upon stepwise addition of Cu^{2+} ions in HEPES buffer (10 mM, pH 6). Excitation wavelength: 508 nm. Dequenching emission spectra of PDI-HIS (0.66 μM) + Cu^{2+} (1.33 μM) upon the stepwise addition of different amounts of ATP in HEPES buffer (10 mM, pH 6).

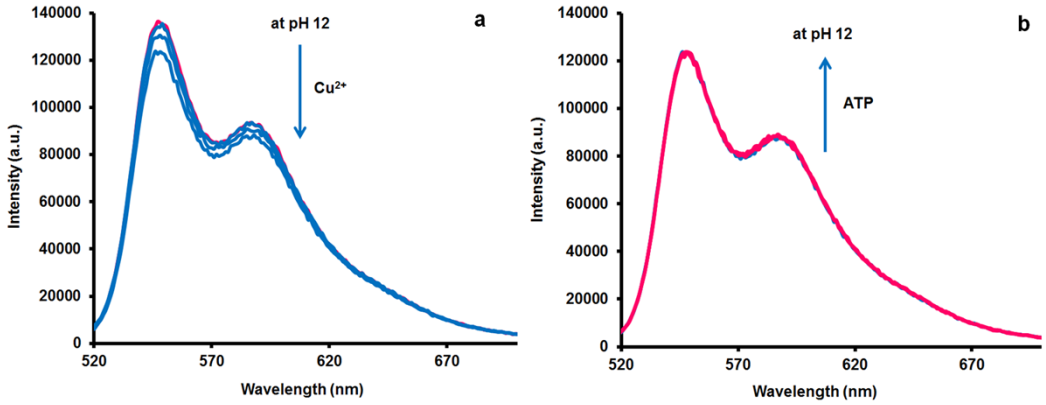

Figure S11 (a) Emission spectra of PDI-HIS (0.66 μm) upon stepwise addition of Cu^{2+} ions in HEPES buffer (10 mM, pH 12). Excitation wavelength: 508 nm. Dequenching emission spectra of PDI-HIS (0.66 μM) + Cu^{2+} (1.33 μM) upon the stepwise addition of different amounts of ATP in HEPES buffer (10 mM, pH 12).

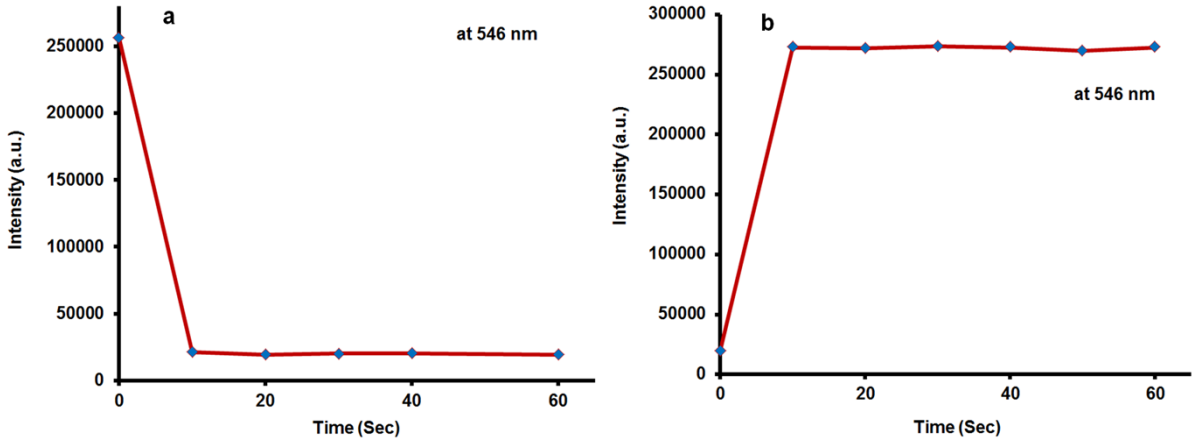

Figure S12 (a) Response time curve for Cu^{2+} evaluated through fluorescence spectra of PDI-HIS (0.33 μm) upon addition of 2 equiv of Cu^{2+} in HEPES buffer (10 mM, pH 7.4). (b) Response time curve for ATP evaluated through fluorescence spectra of PDI-HIS (0.33 μM) + Cu^{2+} (1.33 μM) upon addition of ATP in HEPES buffer (10 mM, pH 7.4).

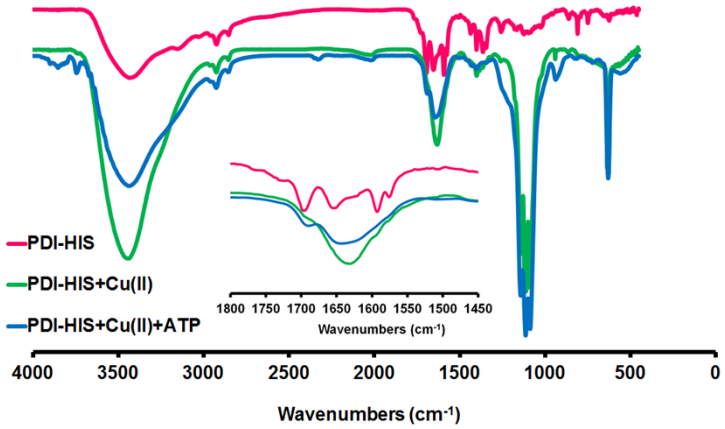

Figure S13 FTIR spectra of PDI-HIS, PDI-HIS+Cu^{2+} and PDI-HIS+Cu^{2+} + ATP.

Detection limit for Cu^{2+}:

The detection limit was calculated on the basis of the fluorescence titration. The fluorescence emission spectrum of PDI-HIS was measured 10 times, and the standard deviation of blank measurement was achieved. To get the slope, the ratio of the emission intensity at 546 nm was plotted as a concentration of Cu^{2+}. The detection limit was calculated using the following equation

$$\text{Detection limit} = 3\sigma/k \quad (1)$$

Where σ is the standard deviation of blank measurement, and k is the slope between the ratios of emission intensity versus [Cu^{2+}].

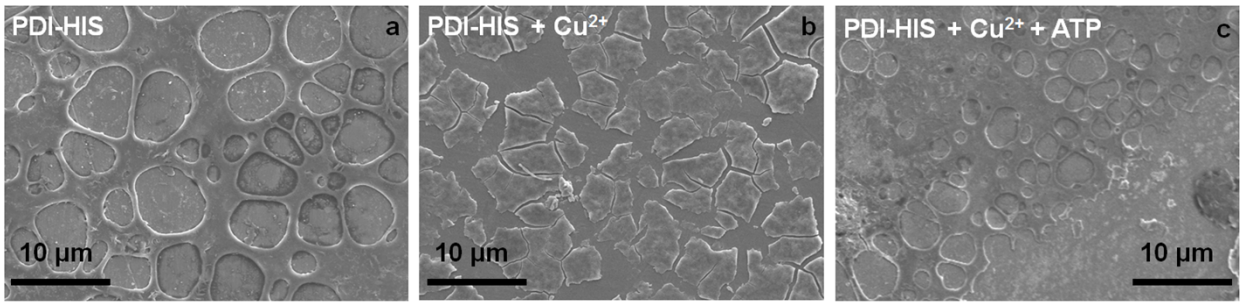

Figure S14 SEM images of PDI-HIS, PDI-HIS+Cu^{2+} complex and PDI-HIS+Cu^{2+} + ATP.

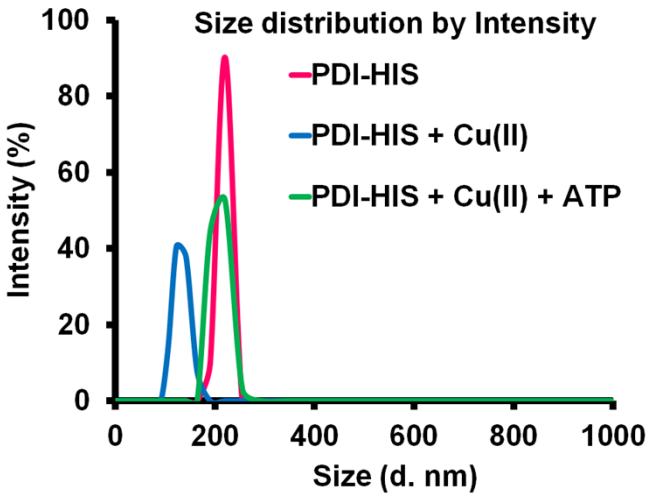

Figure S15 DLS-based particle size analysis of PDI-HIS, PDI-HIS+Cu^{2+} and PDI-HIS+Cu^{2+} + ATP in HEPES buffer at pH 7.4.

Cell culture experiments

A549: human lung cancer, B16: mouse melanoma cell line, was purchased from ATCC and was cultured in DMEM (Dulbecco's Modified Eagle Medium) media supplemented with 5% L-glutamine, 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin-streptomycin) in a humidified 5% CO_2 incubator at 37 ^{0}C for cell viability experiments, fluorescence imaging and bio-sensing studies. HUVECs: human umbilical vein endothelial cells were cultured in EBM complete media using 100 mm tissue culture plates at 37 ^{0}C and 5% CO_2 for 24 h. The human umbilical vein cell line (EA.hy926) was cultured in DMEM complete media. The cells were incubated with various concentrations of PDI-HIS (10µg/ml - 750 µg/ml) for *in vitro* cytotoxicity experiments. PDI-HIS (2 µg/mL in water (pH=7.4)) was kept under UV irradiation inside the cell culture hood for 15-20 mins before use.

Cell viability assay (MTT)

Viability of HUVEC, EA.hy926, A549 and B16 cells were checked by MTT assay according to the published protocol.[1] Initially, 10,000 cells/ well were seeded in per well of 96 well and various concentrations of PDI-HIS (10µg/ml - 750 µg/ml) for cytotoxicity experiment for 24 hours as a dose dependent manner. After 48 hours of treatment 1 mL of MTT stock solution (concentration 5 mg/ mL) was diluted to 10 mL solution using DMEM media and 100 µL of this MTT solution (10 µL 5 mg/ mL MTT + 90 µL of corresponding media) was added to each well by replacing the media and allowed to incubate for 4 h. After 4 h, the media in each well was replaced by 100 µL of DMSO-Methanol mixture (1:1 volume ratio) for solubilizing the violet crystal and kept the mixture on the shaker for homogeneous mixture. Finally, the

absorbance of the mixture was measured at 570 nm using a microplate reader (Varioskan Flash). All the experiments were carried out in triplicate and the results are expressed as normalized viability= {1/$Abs_{\lambda=570}$ (untreated $cells_{blank}$)}×{$Abs_{\lambda=570}$ (treated cells-blank)}.

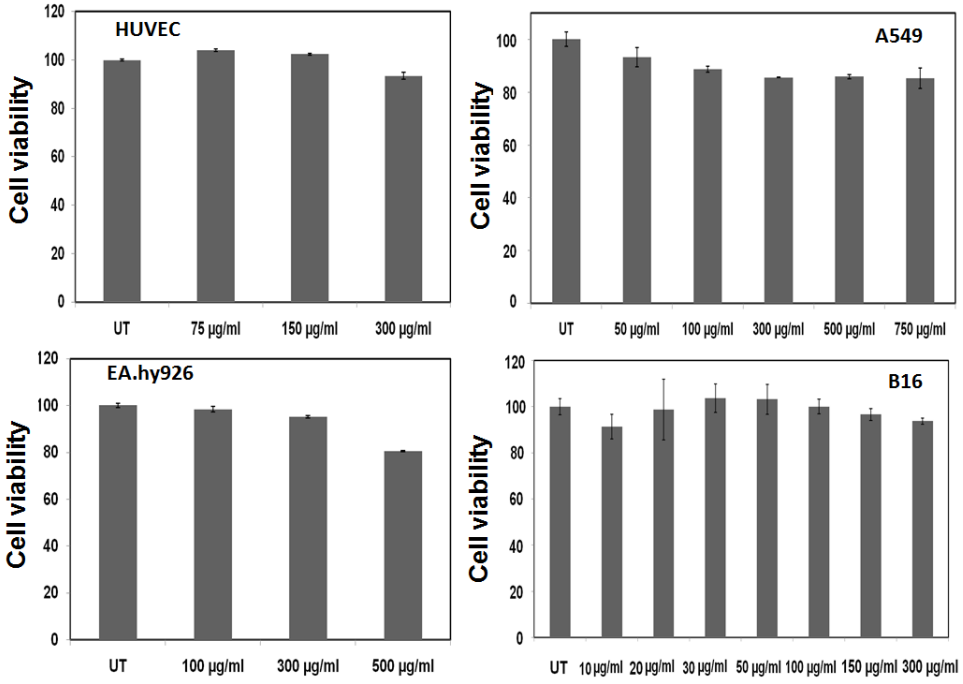

Figure S16 Cell viability assay of PDI-HIS with different cell lines normal (HUVEC & EA.hy926) and cancer cells (A549 & B16) at different concentrations.

Fluorescence cell imaging Study

$2x10^4$ numbers of A549 cells were seeded in each of the 24 well plates and incubated at 37 ^{0}C for overnight in a CO_2 incubator prior to cell imaging studies. On the next day A549 cells were treated with 300 µg/mL and 500 µg/mL of PDI-HIS and incubated for 24 hours. Cells were washed with PBS (4-5 times) then the cells were kept in HBSS buffer (pH=7.4) and finally the fluorescence images were taken by fluorescence microscopy (Nikon Eclipse TE2000-E).[2,3] The red fluorescence images were collected with a 10X & 20X microscope objective with excitation wavelength range λ_{Ex} = 510-560 nm (green) and λ_{Em} = 605 nm (red). A549 cells were then treated with 10^{-3} M (1 mM) of Cu^{2+} for 3 hours. After 2-3 times PBS washing again the fluorescence images were taken in the red filter for copper sensing experiments. Further, the copper treated A549 cells were treated with ATP (20 mM) and incubated for 45 mins-1 hour. The re-enhancement of fluorescence signals from A549 cells due to the incubation of ATP was observed under same excitation-emission filter (red).

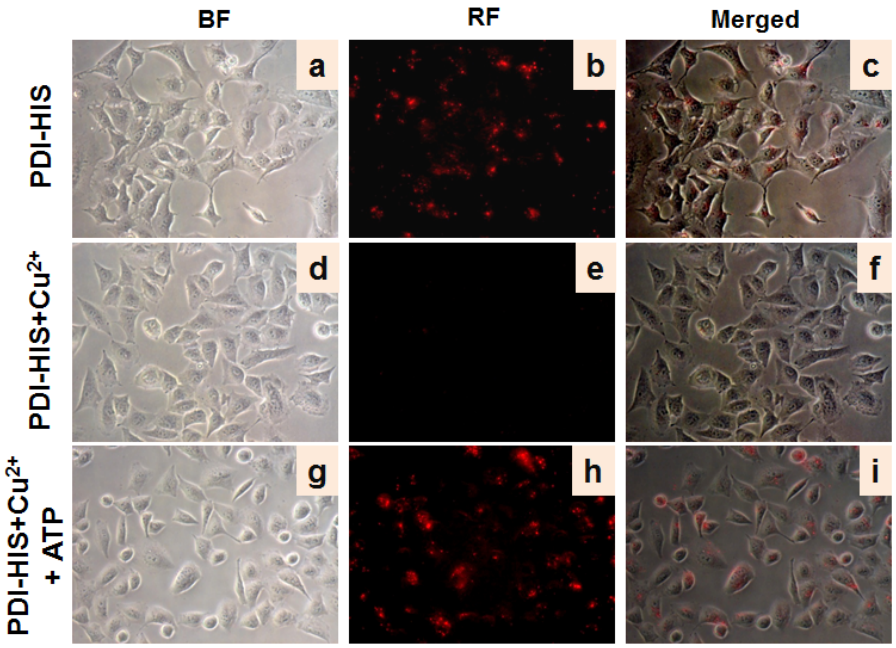

Figure S17 (a, d, g) Bright-field image of A549 cells treated with PDI-HIS, PDI-HIS+Cu^{2+} and PDI-HIS+Cu^{2+} with ATP; (b) Fluorescence image of A549 cells incubated with300 μg/mL of PDI-HIS for 24 h (e) and then further incubated PDI-HIS with 1 mM Cu^{2+} for 3 h; (h) PDI-HIS+Cu^{2+} complex was further incubated with 20 mM ATP for 1 h; (c, f, i). Merged images of A549 cells treated with PDI-HIS, PDI-HIS+Cu^{2+} and PDI-HIS+Cu^{2+} with ATP. Excitation wavelength range λ_{ex} = 510-560 nm (green) and λ_{em} = 605 nm (red), (Nikon eclipase TE 2000-E, 20X magnification).

References

1. P. T. Chou, G. R. Wu, C. Y. Wei, C. C. Cheng, C. P. Chang, F. T. Hung. *J. Phys. Chem. B.* 2000, **104,** 7818-7829.

2. S. Mukherjee, D. Chowdhury, R. Kotcherlakota, S. Patra, B. Vinothkumar, M. P. Bhadra, B. Sreedhar and Patra, C. R. *Theranostics* 2014, **4,** 316-335.

3. A. Modak, A. K. Barui, C. R. Patra and Bhaumik, A. *Chem. Commun.* 2013, **49,** 7644-7646.